

## **An Electrochemical Sensor for the Detection of Unmodified Nucleic Acids**

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The detection of nucleic acid hybridized to a complementary capture probe is of central importance in genomic research and pathogen diagnostics. Current methodologies require the covalent attachment of an enzymatic, fluorescent, chemiluminescent or radioactive label in order to detect the hybridized target. We have developed a direct electrochemical detection method for DNA and RNA that does not require covalent attachment of reporter molecules. In this method, oligonucleotide probes are bound to a tin-doped indium oxide (ITO) electrode via a silane or self-assembled phosphonate linkage. Upon hybridization of the complementary target nucleic acid, the hybrid is detected using a redox-active mediator, tris(2,2'-bipyridyl) ruthenium(II) [Ru(bpy)<sub>3</sub><sup>2+</sup>]. When the appropriate potential is applied, the mediator oxidizes guanine residues via a catalytic cycle and the current produced measures the amount of guanine hybridized at the electrode which, in turn reflects the amount of specific nucleic acid in the sample. The oxidation of guanine by Ru(bpy)<sub>3</sub><sup>2+</sup> can be utilized for the detection of single and double stranded DNA, as well as RNA without the need for reverse transcription to form cDNA. This method, unlike other electrochemical techniques, does not require the target nucleic acid to be in close proximity or directly wired to the electrode. It also does not require the use of duplex specific, redox-active indicators.

The format currently in use is a 200-μm diameter ITO electrode, upon which a nucleic acid capture probe is assembled. Substitution of hypoxanthine for guanine in the capture probe minimizes the background current generated by the capture probe, because hypoxanthine is 1000 fold less electrochemically reactive than guanine. Using a 200-μm diameter electrode, we have successfully detected hybridization of mRNA out of cell lysate. The specificity and sensitivity of the system continues to be optimized using nucleic acid capture probes and targets, PCR products and mRNA from cultured cells.

The detector system can be readily multiplexed and to that end, we have designed a 96 well microtiter plate with seven 200-μm ITO working electrodes per well to be used in gene expression analysis. The electrode surface was characterized by electrochemistry, atomic force microscopy, and X-ray photoelectron spectroscopy. Cyclic voltammetry and chronoamperometry were used for system interrogation. The correlation between the electrochemical performance of ITO and its microstructure such as crystallinity, film thickness, surface roughness, tin concentration, and crystallographic texture was established through a range of conditions used during the ITO deposition.